

Predicting the activity of the natural phytotoxic diphenyl ether cyperine using Comparative Molecular Field Analysis[†]

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Abstract: Protoporphyrinogen oxidase (Protox) is the target site of a large number of commercial herbicides. Previous QSARs performed at a two-dimensional level reproduced the activity of individual data sets relatively well, but these models could not be used to predict the activity of structurally related derivatives. We have developed a more reliable model by applying three-dimensional molecular techniques to a set of 31 phenyl ether analogues. Inhibitory activity at the molecular site of action was chosen because it circumvents the effects of uptake, translocation and metabolism of the compounds occurring in whole-plant studies. Increased predictability was achieved by aligning the diphenyl ether analogues along the trifluoromethyl phenyl ($q^2=0.70$) ring rather than along the nitrophenyl ($q^2=0.65$) ring or along the centroids ($q^2=0.69$). This new analysis differentiated between *R* and *S* enantiomers and allowed the prediction of the inhibitory activity of the natural diphenyl ether cyperine. The prediction model suggests that the binding of cyperine on the active site of Protox is stereospecific.

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Keywords: natural products; diphenyl ether; QSAR; CoMFA; protoporphyrinogen oxidase; Protox; herbicide

1 INTRODUCTION

Diphenyl ether (DPE) inhibitors of protoporphyrinogen oxidase (Protox) have been a successful class of herbicides. The relatively weak DPE herbicide nitrofen was introduced in the early 1960s and numerous DPE analogues that are potent inhibitors of Protox have been synthesized since then.^{1,2} Many of these DPE analogues are (or have been) successful commercial herbicides.¹ Structure-activity relationship (SAR) studies on DPE herbicides published previously^{3–10} used whole-plant biological activity. However, *in vivo* biological activity data are not useful if one is interested in understanding the relationship between structural characteristics of inhibitors and their molecular binding site. At the whole-plant level, other factors such as uptake, translocation and metabolism contribute to the overall activity of Protox inhibitors.^{11–14} As a result, the most herbicidally active compounds may not necessarily be the most potent Protox inhibitors (eg Dayan *et al*¹⁵). Conversely, strong Protox inhibitors may have poor herbicidal activity.¹⁶

The molecular site of action of DPE herbicides was discovered in 1989¹⁷ and is now known to be the target of thousands of compounds, including many heterobicyclic structures.^{13–15,18–21} Several two-dimensional (2-D) quantitative structure-activity relationship (QSAR) studies have correlated DPE inhibition with

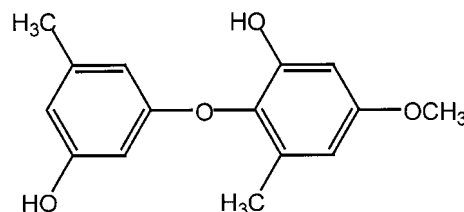


Figure 1. Cyperine, a natural phytotoxic DPE isolated from several species of fungi $pl_{50}=4.22$.

Protox inhibition.^{5,6,22,23} However, these studies are limited in that they cannot distinguish active from inactive enantiomers and could not predict the activity of cyperine (a natural diphenyl ether produced by pathogenic fungi of *Cyperus rotundus* L.) (Fig. 1). A more recent study by Durst²⁴ using a set of 24 diphenyl ethers demonstrated the benefit of three-dimensional (3-D) techniques such as Comparative Molecular Field Analysis (CoMFA) in determining QSAR between compounds.

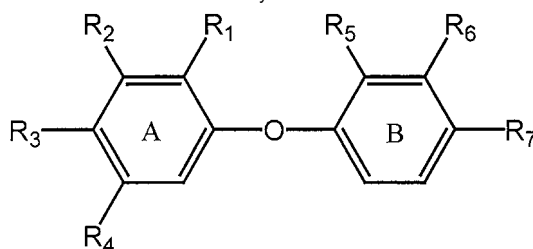
We are interested primarily in the mode of action of natural products and how they interact with their target sites at the molecular level. When the shape of a ligand binding pocket is not characterized, such as with Protox, we must rely on 3-D QSAR information using inhibitory data to glimpse at the ligand-receptor interaction. We present a 3-D approach that describes

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Table 1. Chemical structures of diphenyl ether derivatives used in this study**Compounds 1–20**

Compound	Herbicide	R_1	R_2	R_3	R_4	R_5	R_6	R_7	pl_{50}
1	PPG-1055	Cl	H	CF ₃	H	H	C(CH ₃)=NOCH ₂ COOH	NO ₂	8.10
2	PPG-1013	Cl	H	CF ₃	H	H	C(CH ₃)=NOCH ₂ COOCH ₃	NO ₂	7.92
3	MT-124	Cl	H	CF ₃	H	H	—O—Furan	NO ₂	7.82
4	Oxyfluorfen	Cl	H	CF ₃	H	H	OC ₂ H ₅	NO ₂	7.60
5	Benzofluorfen	Cl	H	CF ₃	H	H	COOCH ₂ COOH	NO ₂	7.52
6	RH-4638 (<i>R</i>)	Cl	H	CF ₃	H	H	OCH(CH ₃)COOC ₂ H ₅	NO ₂	7.46
7	Lactofen	Cl	H	CF ₃	H	H	COOCH(CH ₃)COOC ₂ H ₅	NO ₂	7.40
8	Acifluorfen-Me	Cl	H	CF ₃	H	H	COOCH ₃	NO ₂	7.40
9	MC-15608	Cl	H	CF ₃	H	H	COOCH ₃	Cl	7.40
10	Nitrofen	Cl	H	Cl	H	H	H	NO ₂	7.30
11	Nitrofluorfen	Cl	H	CF ₃	H	H	H	NO ₂	7.22
12	Bifenox	Cl	H	Cl	H	H	COOCH ₃	NO ₂	7.22
13	Fluorodifen	NO ₂	H	CF ₃	H	H	H	NO ₂	6.74
14	RH-4639 (<i>S</i>)	Cl	H	CF ₃	H	H	OCH(CH ₃)COOC ₂ H ₅	NO ₂	5.46
15	RH-5348	Cl	H	H	CF ₃	H	COOCH ₃	NO ₂	5.41
16	Acifluorfen	Cl	H	CF ₃	H	H	COOH	NO ₂	5.40
17	RH-8378	H	CH ₃	H	H	H	H	NO ₂	4.22
18	Aclonifen	H	H	H	H	Cl	NH ₂	NO ₂	3.98
19	RH-0211	H	H	H	H	H	H	NO ₂	3.66
20	RH-5349	Cl	H	H	CF ₃	H	COOH	NO ₂	3.38

the spatial characteristics of 31 DPEs required for Protox-inhibiting activity at the molecular level compiled from two sets of DPE analogues for which the Protox inhibitory activity is known.^{5,7} The models generated were used to predict the biological activity of the natural DPE cyperine on Protox.

2 3-D QSAR ANALYSIS

QSAR methods have been successful at characterizing the activity of structurally related compounds because biological activity can, in many instances, be correlated with structural features of ligands. Steric and electrostatic parameters are most commonly used in QSAR because interactions between herbicides and the receptor binding sites usually involve non-covalent binding.

The recent development of 3-D QSAR methods has greatly enhanced the molecular analyses that were previously possible with classical QSAR methods. One of those methods, CoMFA, developed by Tripos Inc (St Louis, MO, USA),²⁵ was used in this study.

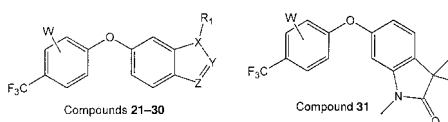
2.1 Methodology

Two sets of molecules designed on the DPE backbone were selected for this study because their I_{50} values on Protox were available. The inhibitory activity of the herbicides was obtained spectrofluorometrically by

measuring the effect of the compounds on Protox activity in barley etioplasts. The first set consisted of 20 DPEs (Table 1) that included several commercial products (eg **4**, **5**, **7**, **8**, **10–13**, **16** and **18**).⁵ The second set consisted of 11 experimental DPE-derived molecules (Table 2) that have a heterocyclic ring fused to one of the phenyl rings.⁷ The activity of cyperine, a herbicidally active natural DPE extracted from several fungal plant pathogens,^{26–28} was predicted using the CoMFA models derived in this analysis.

2.1.1 Building of structures

A X-ray crystal structure of acifluorfen (**16**) obtained from the Cambridge Structure Database²⁹ served as a template on which all the analogues were built. The torsion angles around the ether linkage of the x-ray structure of **16** were assigned as a common starting point for all of the analogues used in this study. As a rule, side chains of the analogues were constructed in *trans*- rather than *cis*-configuration using Sybyl 6.3 (Tripos Associates, St Louis, MO, USA) on a Silicon Graphics Indigo 2 Impact (250MHz Mips IP22 R4400/4010). Gasteiger–Hückel charges were calculated and the molecules were minimized using Tripos force field to obtain low-energy conformers. Minimization was initiated with Simplex (a non-derivative based procedure) for 100 iterations, followed by Powell (a first-derivative based method) for 1000



Compound	W	X	Y	Z	R ₁	pI ₅₀
21	2-F, 6-Cl	N	N	N	CH ₂ COOCH ₃	7.15
22	2-Cl, 6-F	CH	N	O	CH ₂ COOCH ₃	7.26
23	2-Cl, 6-F	CH	N	O	OCH ₂ COOCH ₃	6.60
24	2-F, 6-Cl	N	N	CH	CH ₂ COOCH ₃	7.26
25	2-F, 6-Cl	N	CO	O	CH ₂ COOCH ₃	7.45
26	2-F, 6-Cl	CH ₂	CO	NCH ₂ CH ₃	—	6.60
27	2-F, 6-Cl	C	CO	NCH ₃	—CH ₂ CH ₂ —	7.52
28	2-F, 6-Cl	C	CO	NCH ₂ C≡CH	—CH ₂ CH ₂ —	7.15
29	6-Cl	C	CO	NCH ₃	—CH ₂ CH ₂ —	6.82
30	6-Cl	CH	N	O	CH ₂ COOCH ₃	6.19
31	2-F, 6-Cl	—	—	—	—	7.15

Table 2. Chemical structures of the benzheterocyclic derivatives used in this study

iterations, until convergence criteria of 0.005 kcal mol⁻¹ were met.

2.1.2 Gridsearch analysis

Since the bonds involved in the ether linkage between the two phenyl rings have free rotation, a gridsearch was performed and the energy of each rotational isomer was calculated to determine the global minimum of acifluorfen (**16**). The gridsearch analysis was performed for the rotation around the bond between the trifluoromethyl phenyl ring and the ether oxygen. Conformers were generated in 5° increments from 0° to 355° and the energy of each conformer was calculated using Tripos force field until convergence criteria of 0.01 kcal mol⁻¹ were reached.

2.1.3 Assignment of centroids

Centroids are 'dummy' atoms that represent the theoretical center of ring structures. Centroids were assigned independently for each phenyl ring of the DPE structures. All six atoms for each phenyl ring were used to assign the centroid. This technique permits the alignment of the DPE rings based on the center of each ring, while allowing for differences between angles of the planes of each ring.

2.1.4 Alignment of molecular databases

Acifluorfen (**16**) was oriented using the BEST VIEW feature of Sybyl. This molecule served as a template for aligning the remaining structures in the databases. Since the precise nature of the interaction between the DPE rings and the receptor site on Protox is not known, one must determine if both rings are involved in the binding or whether one ring plays a more important role than the other. Therefore, three different alignment rules were generated for CoMFA analyses. The first database consisted of DPE molecules aligned on the plane of the nitrophenyl rings (Plate 1A), the second database was aligned on the centroids and the ether oxygen (Plate 1B), and the

third database consisted of DPE aligned on the plane of the trifluoromethyl phenyl rings (Plate 1C).

2.1.5 CoMFA and prediction of the inhibitory activity of cyperine

All analyses were performed using the default lattice parameters for CoMFA consisting of a 3-D grid with a width of 2 Å. CoMFA was performed using the default probe consisting of a sp³ carbon with a +1 charge. Attempts to improve the CoMFA by transforming the lattice grid into a face-cubic pattern and using other probes (eg N, S and O) were not successful. The biologically active natural diphenyl ether cyperine was submitted to each model under two different orientations. This was necessary because cyperine does not possess either nitrophenyl or trifluoromethyl phenyl rings. Thus, we generated two possible alignments of cyperine for each model. Alignment **a** consisted of the ring with the methoxy substituent toward the nitrophenyl ring and alignment **b** consisted of the ring with the methoxy substituent toward the trifluoromethyl phenyl ring.

3 RESULTS AND DISCUSSION

3.1 Gridsearch analysis

The conformational search performed on the rotation of the bond connecting the trifluoromethyl phenyl ring to the ether oxygen indicated that DPE structures are not rigid. Changing the torsional angle on the trifluoromethyl phenyl side of the ether bridge affects the torsional angle of the other ether bond (Plate 2A and 2B). The two phenyl rings appear to repulse each other when the torsion between trifluoromethyl phenyl ring and the ether oxygen is -15, causing the nitrophenyl ring to flip around (Plate 2B) but this does not result in a significant change in the overall energy of the molecule (data not shown). Since the bonds on either side of the ether oxygen appear to rotate freely, no absolute conformation could be determined. Therefore, the conformation derived

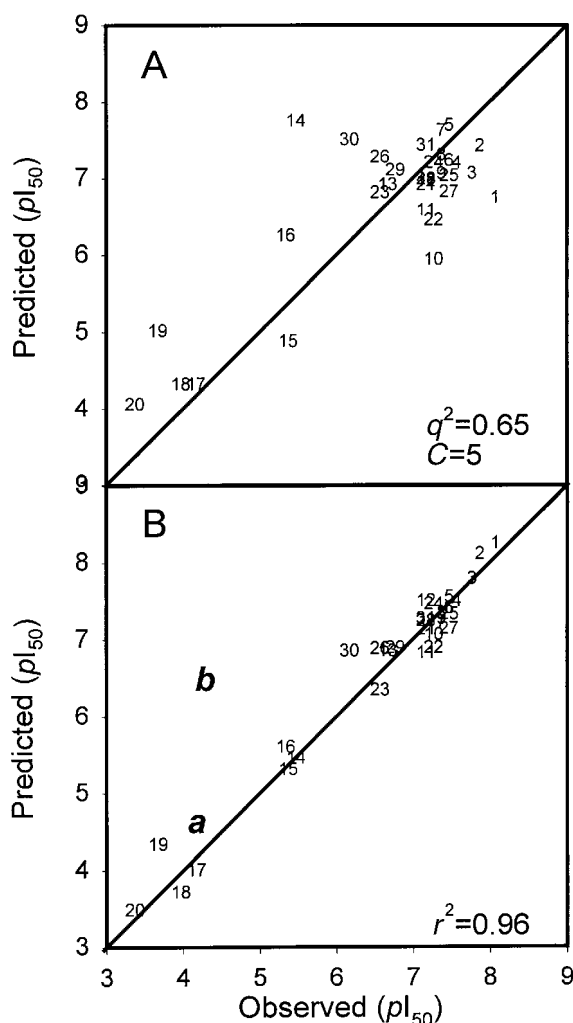


Figure 2. Graphs of CoMFA results from the nitrophenyl-aligned model showing (A) predicted values from the cross-validated partial least square (PLS) analysis, and (B) predicted values from the non-validated PLS analysis showing better prediction of cyperine with orientation **a** than orientation **b**.

from the X-ray structure of **16** (as indicated by an asterisk on Plate 2B) was assumed to represent the conformation of this compound adequately.

3.2 Importance of the alignment rules

Alignment played an important role in generating prediction models. While all q^2 values generated by the models were reasonably good, the values increased from structures aligned on the nitrophenyl rings ($q^2=0.65$), to the set aligned on the centroids ($q^2=0.69$), to the set aligned on the trifluoromethyl phenyl rings ($q^2=0.70$) (Figs 2A–4A). The predicted biological activities of Protox inhibitors **19** and **30** were outside the 95% confidence interval in the models generated with either the nitrophenyl-aligned (Fig. 2B) or the centroid-aligned (Fig. 3B) datasets, although the 95% confidence was narrower with the nitrophenyl alignment than with the latter model. Prediction of Protox inhibition was best approximated with the dataset aligned on the trifluoromethyl phenyl rings (Fig. 4B). The 95% confidence interval was

small, relative to the other two models, and the biological activity of all the compounds included in the dataset were predicted within the 95% confidence interval (Fig. 4B).

The fact that the trifluoromethylphenyl ring-aligned set yielded a more accurate prediction model may be an important observation. Most of the second generation oxygen-bridged Protox inhibitors have been designed on the DPE backbone which retained the trifluoromethylphenyl group instead of the nitrophenyl ring.³⁰ This suggests that the orientation of the plane of the trifluoromethyl phenyl ring plays an important role in binding to Protox.

Dayan *et al*³⁰ reported a similar trend using a set of 21 DPEs. Their prediction model generated better q^2 values when the structures were aligned on the trifluoromethyl phenyl rather than on the nitrophenyl rings. However, q^2 was smaller than the value obtained in this study when the model did not include log *P* values in the CoMFA analysis (eg, 0.37 and 0.45 for the nitrophenyl and trifluoromethyl phenyl rings,

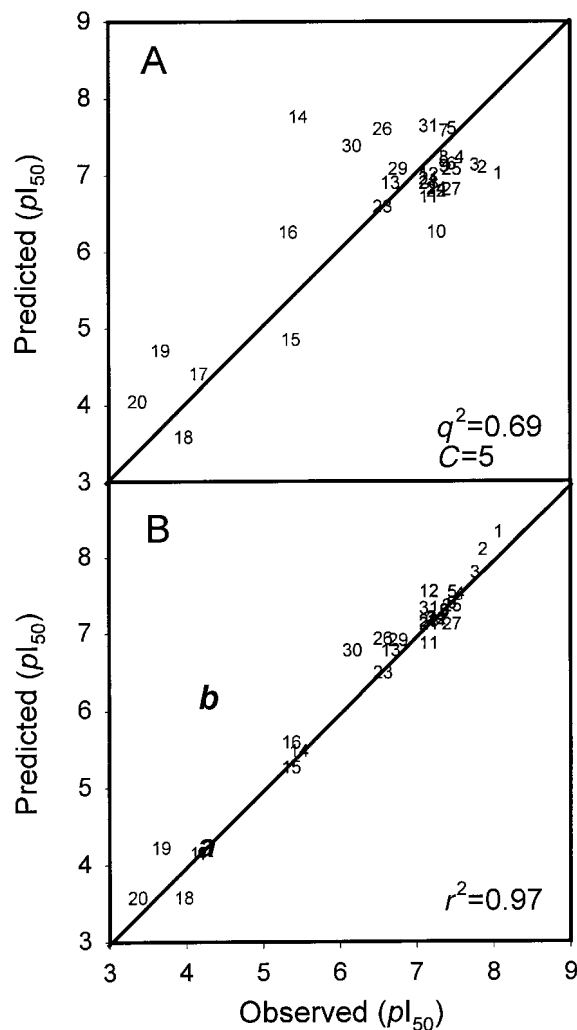


Figure 3. Graphs of CoMFA results from the centroid-aligned model showing (A) predicted values from the cross-validated partial least square (PLS) analysis, and (B) predicted values from the non-validated PLS analysis showing better prediction of cyperine with orientation **a** than orientation **b**.

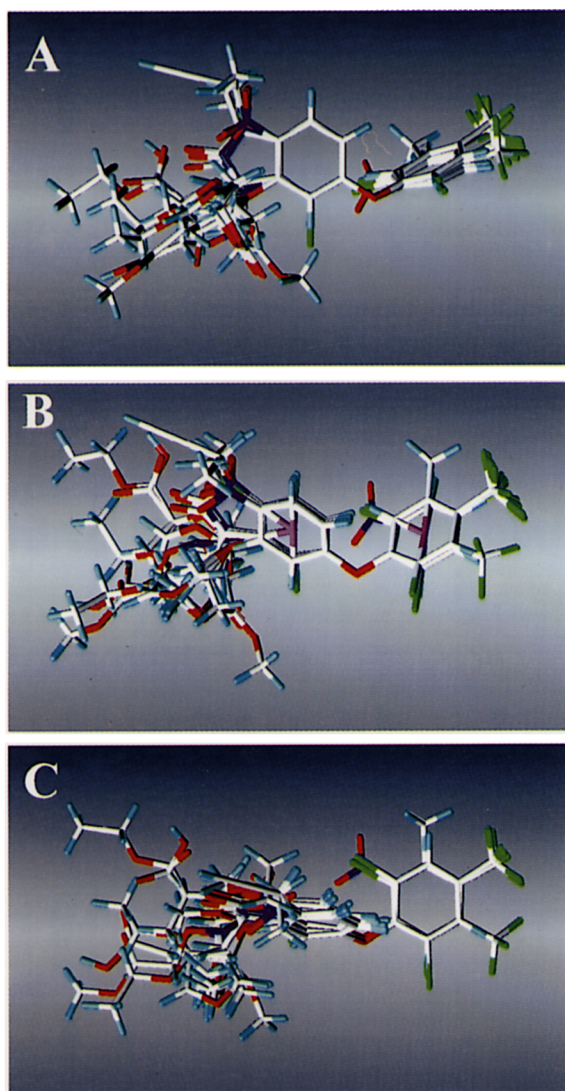


Plate 1. Overlay of the 31 DPE molecules used in this study. The three alignment rules applied were (A) on their nitrophenyl ring, (B) on the centroids and the ether oxygen and (C) on the trifluoromethyl phenyl ring.

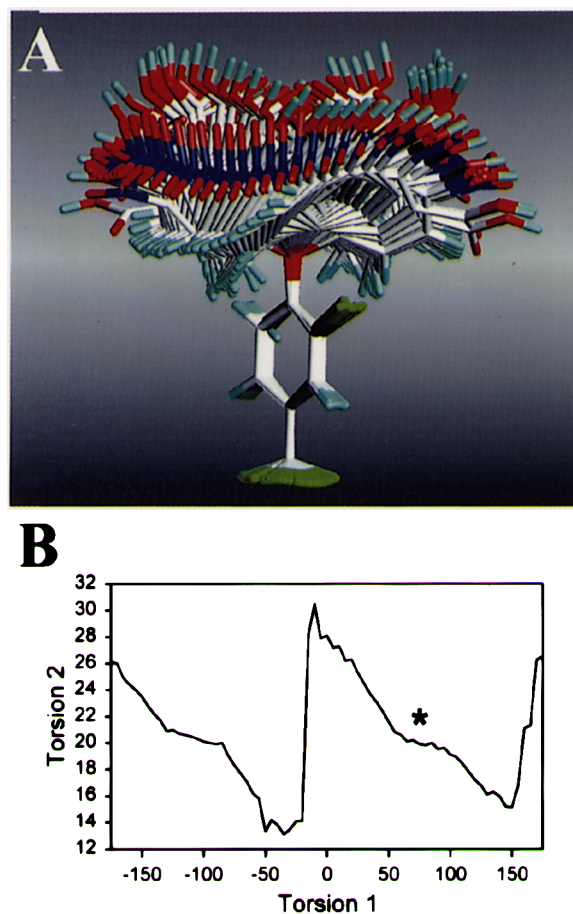


Plate 2. Gridsearch at the bond between the trifluoromethyl phenyl ring and the ether oxygen. (A) Overlay of 72 rotational isomers aligned on the trifluoromethyl phenyl ring; the rotation around the adjacent ether bond is not restricted. (B) Changes in torsional angle of the nitrophenyl ring as affected by the rotation around the ether-trifluoromethyl phenyl ring linkage. The asterisk represents the conformation of the x-ray structure of **16**.

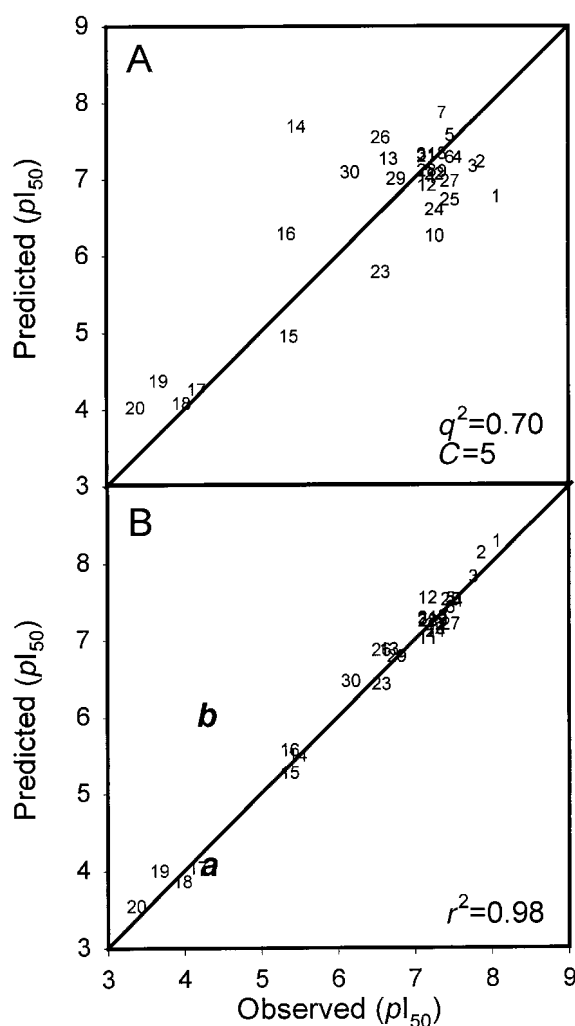


Figure 4. Graphs of CoMFA results from the trifluorophenyl-aligned model showing (A) predicted values from the cross-validated partial least square (PLS) analysis, and (B) predicted values from the non-validated PLS analysis showing better prediction of cyperine with orientation a than orientation b.

respectively). Including log P values improved the models to levels similar to the ones obtained in our current study. Including log P in the models generated with the three sets of alignments used in this study would probably have had a similar enhancement effect. Unfortunately, log P values were not available for one of the datasets,⁷ and the calculated log P values (using semi-empirical methods) could not be used because they were significantly different from the known log P values of some of the compounds (data not shown).

3.3 Prediction of enantiomeric structures

Enantiomeric pairs of Protox inhibitors have previously been poorly predicted by traditional techniques because 2-D representations of these molecules are identical. Therefore, differences in activity associated with stereospecific orientation of the enantiomeric moieties cannot be explained by such traditional means,⁵ and one of the enantiomers has to be deleted from the dataset because it behaves as an outlier. 3-D

QSAR circumvents this problem because stereospecific differences between enantiomers can be distinguished in 3-D space. In this respect, this study is a major improvement over previous QSAR studies of Protox inhibitors. The enantiomers **6** and **14** (Table 1) were effectively predicted by all three models (Figs 2, 3 and 4).

3.4 Prediction of cyperine activity by various models

Since all three models predicted Protox inhibitory activity of the diphenyl ether dataset successfully, we submitted cyperine in each model and queried for its predicted biological activity. The pI_{50} of cyperine is 4.22,³¹ and the predicted values for both alignments of cyperine are indicated as **a** and **b** on Figs 2–4. Alignment **a** was consistently better than **b**, and in fact, the predicted values of alignment **b** were so far from the observed values that the residual values were beyond the scale of the residual plots (data not shown). These data indicate that the binding of cyperine on Protox may be quite stereospecific. All three models predicted the biological activity of the **a** alignment satisfactorily. However, unlike with synthetic Protox inhibitors, the best prediction was obtained with the model generated using the centroid (Fig. 3B) rather than the datasets aligned on the trifluoromethyl phenyl ring (Fig. 3A).

4 CONCLUSIONS

QSAR modeling of Protox inhibitors has become more refined as molecular modeling and statistical software have advanced. Steric, electronic, chemical and physiochemical parameters can be calculated quickly and correlated accurately to better predict biological activities. Activity of DPE structures suspected to be Protox inhibitors can be approximated with confidence using our 3-D models, even differentiating between enantiomers whose biological activities differ significantly.

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